

Experimental evidence of fragile-to-strong dynamic crossover in DNA hydration water

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We used high-resolution quasielastic neutron scattering spectroscopy to study the single-particle dynamics of water molecules on the surface of hydrated DNA samples. Both H₂O and D₂O hydrated samples were measured. The contribution of scattering from DNA is subtracted out by taking the difference of the signals between the two samples. The measurement was made at a series of temperatures from 270 down to 185 K. The relaxing-cage model was used to analyze the quasielastic spectra. This allowed us to extract a Q -independent average translational relaxation time $\langle\tau_T\rangle$ of water molecules as a function of temperature. We observe clear evidence of a fragile-to-strong dynamic crossover (FSC) at $T_L=222\pm 2$ K by plotting $\log\langle\tau_T\rangle$ versus T . The coincidence of the dynamic transition temperature T_c of DNA, signaling the onset of anharmonic molecular motion, and the FSC temperature T_L of the hydration water suggests that the change of mobility of the hydration water molecules across T_L drives the dynamic transition in DNA. © 2006 American Institute of Physics. [DOI: 10.1063/1.2372491]

I. INTRODUCTION

It is known that hydrated biomacromolecules show sharp slowing down of their functions (kinetics of biochemical reactions) within a temperature interval $T\sim 250\text{--}200$ K.¹ It was also found, from neutron and x-ray scattering or from Mössbauer spectroscopy, that the measured mean-squared atomic displacement $\langle x^2 \rangle$ of the biomolecules exhibits a sharp rise in the same temperature range.^{1–5} This sharp increase in $\langle x^2 \rangle$ was taken as a sign for a dynamic transition (or sometimes called glass-transition) in the biomolecules occurring within this temperature range. In most of these papers, the authors suggest that the transition is due to a strong rise of anharmonicity of the molecular motions above this transition temperature.¹ Later on, it was demonstrated that the dynamic transition can be suppressed in dry biomolecules,² or in biomolecules dissolved in trehalose.⁵ Moreover, it can be shifted to a higher temperature for proteins dissolved in glycerol.⁴ Thus the dynamic transition can be controlled by changing the surrounding solvent of the biomolecules. On the other hand, it was found some time ago, from Raman scattering that supercooled bulk water has a dynamic crossover transition at 220 K,⁶ similar to that predicted by mode-coupling theory.⁷ Approximate coincidence of these two characteristic temperatures, one for the slowing down of bio-

chemical activities and the sharp rise in $\langle x^2 \rangle$ in biomolecules and the other for the dynamic crossover in water, suggests a relation between the dynamic transition of biomolecules and that of their hydration water.⁸

Another striking experimental fact is that this dynamic transition temperature, as revealed by change of slope in $\langle x^2 \rangle$ versus temperature plot, occurs at a universal temperature range from 250 to 200 K in all biomolecules examined so far. This list includes globular proteins, DNAs, and RNAs. This feature points to the plausibility that the dynamical transitions are not the intrinsic properties of the biomolecules themselves but are imposed by the hydration water on their surfaces.

However, $\langle x^2 \rangle$ (mostly coming from hydrogen atoms) is an integrated quantity of motion, arising from different types of molecular motions: both vibrations and librations of hydrogen atoms with respect to their binding center in the molecules, as well as large amplitude transitions between conformational substates of the macromolecule. Therefore, it is difficult to identify the microscopic processes underlying this transition and to pin-point the actual dynamical transition temperature from the inspection of $\langle x^2 \rangle$ only. On the other hand, dynamical quantities, such as the self-diffusion coefficient, the viscosity, and the structural relaxation time (or the so-called α -relaxation time), could show a sharper transition as a function of temperature and pressure if there is a genuine dynamic transition in the hydration water.

In this paper, we demonstrate decisively using high-

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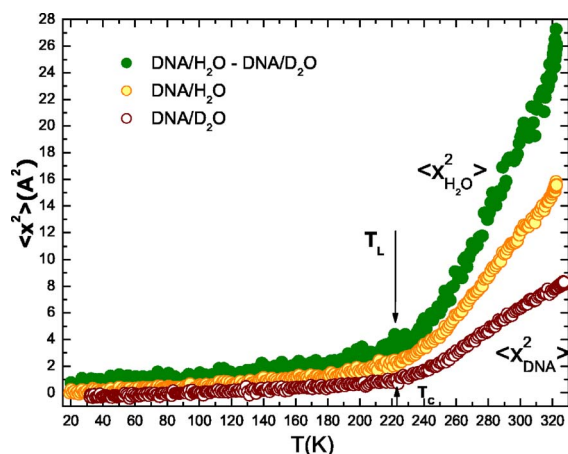


FIG. 1. The mean-squared atomic displacement averaged over all the hydrogen atoms, $\langle x^2 \rangle$, extracted from the Debye-Waller factor measured by an elastic scan, as a function of temperature for the H₂O hydrated (open circles, middle curve without label) and the D₂O hydrated (open circles, lower curve, labeled as $\langle x^2_{\text{DNA}} \rangle$) DNA samples and for the hydration water (solid circles, labeled as $\langle x^2_{\text{H}_2\text{O}} \rangle$) obtained from the difference between the first two. One can clearly see from the top and the bottom curves sharp transition of slope at around 220 K, indicating that the dynamic crossover temperatures of the DNA (T_C) and the hydration water (T_L) are approximately the same.

resolution quasielastic neutron scattering (QENS) spectroscopy that there is a sharp dynamic crossover, identified to be a fragile-to-strong dynamic crossover (FSC), temperature of the hydration water in DNA at $T_L = 222 \pm 2$ K. This change of mobility of the water molecules across T_L drives the dynamic transition in DNA which happens at the same temperature. We have recently found the same dynamic crossover temperature of $T_L = 220$ K for hydration water in protein

lysozyme,⁹ which further supports our conjecture that it is a change of mobility of the hydration water which triggers the dynamic transition in biomolecules.

II. SAMPLE DESCRIPTION AND NEUTRON SCATTERING EXPERIMENT

Highly polymerized (calf thymus) DNA, sodium salt, was obtained from Sigma (D1501, batch number 091k7030) and used without further purification. The sample was extensively lyophilized to remove any water left. The dry DNA fibers were then hydrated isopiastically at 5 °C by exposing them to water vapor in equilibrium with a NaClO₃ saturated water solution placed in a closed chamber (relative humidity, RH=75%). The final hydration level was determined by thermogravimetric analysis and also confirmed by directly measuring the weight of absorbed water. This hydration level corresponding to about 15 water molecules per base pairs was chosen to have the primary hydration sites almost completely filled (i.e., one monolayer of water). This latter condition corresponds to equilibration against RH=80% (Ref. 10) and about 15 water molecules per base pairs.¹¹ A second sample was then prepared using D₂O in order to subtract out the incoherent signal from the DNA hydrogen atoms. Both hydrated samples had the same water or heavy water/dry DNA molar ratio. Differential scanning calorimetry analysis was performed in order to detect the absence of any feature that could be associated with the presence of bulklike water.

High-resolution incoherent QENS spectroscopy method is used to determine the temperature dependence of the average translational relaxation time $\langle \tau_T \rangle$ for the hydration water. Because neutrons are predominantly scattered by an in-

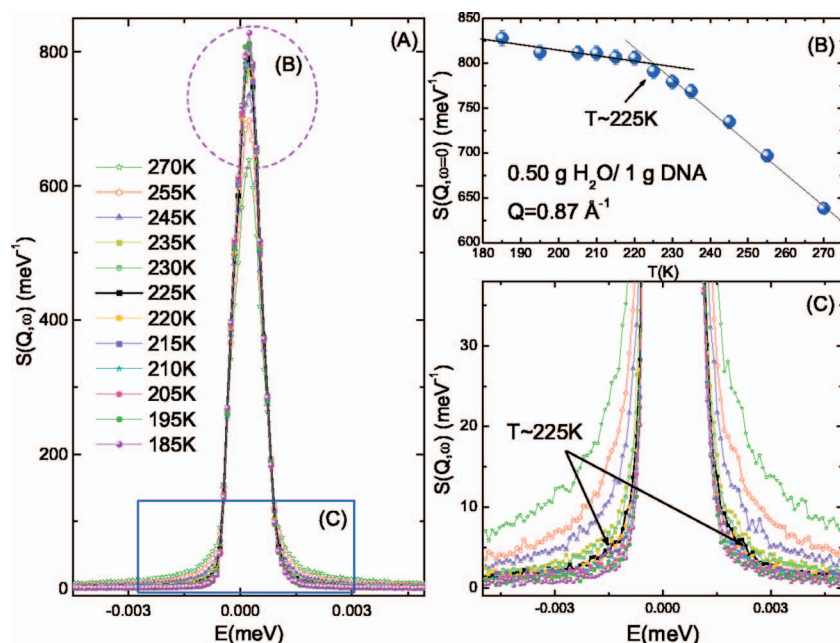


FIG. 2. (Color) The difference neutron spectra between the H₂O hydrated and the D₂O hydrated DNA samples. Panel (A) displays the area-normalized QENS spectra at $Q=0.87 \text{ \AA}^{-1}$ at a series of temperatures. Panels (B) and (C) display the heights of the peak as a function of temperature and the wing spectral region, respectively, at those temperatures. One notes from panel (B) a cusplike transition signaling the rate of change of peak height from a steep high temperature region to a slower low temperature region at a crossover temperature of about 225 K. The error bars are of the size of the data points. In panel (C), we may notice, from the wings of these spectral lines, that two groups of curves, 270–230 K and 220–185 K, are separated by the curve at temperature 220 K. In this panel, the scatter of the experimental points gives an idea of the error bars.

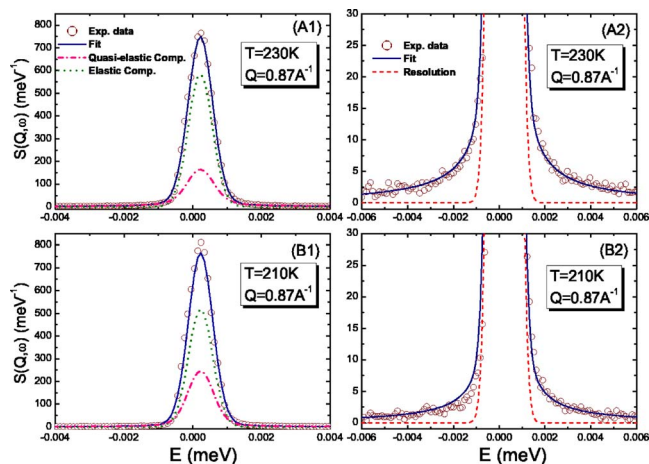


FIG. 3. RCM analyses of the difference QENS spectra at two temperatures, above and below the crossover temperature. Panels (A1) and (B1) show the results of the full analyses of the spectra at two temperatures. Panels (A2) and (B2) indicate the detail fittings of the wings. The resolution function is also indicated in the figure. One can see a sharpening of the quasi-elastic peak as temperature goes below the crossover temperature T_L at 220 K. The scatter of the experimental points gives an idea of the error bars.

coherent process from the hydrogen atoms in water, high-resolution QENS technique is an appropriate tool for the study of diffusional process of water molecules. Using the high-flux backscattering spectrometer (HFBS) in NIST Center for Neutron Research (NCNR), we were able to measure the Q -dependent relaxation time $\tau_T(Q)$ [in Eq. (1)] from ≈ 400 ps to ≈ 5 ns over the temperature range of 270–185 K, spanning both below and above the FSC temperature. For the chosen experimental setup, the spectrometer has an energy resolution of $0.8 \mu\text{eV}$ and a dynamic range of $\pm 11 \mu\text{eV}$,¹² in order to be able to extract the broad range of relaxation times covering both the fragile and the strong regimes of the average relaxation times $\langle\tau_T\rangle$ from measured spectra.

III. RELAXING-CAGE MODEL ANALYSIS

QENS experiments measure the Fourier transform of the intermediate scattering function (ISF) of the hydrogen atoms, $F_H(Q, t)$, of water molecules on the surface of DNA. Molecular dynamics (MD) simulations have shown that the ISF of both bulk¹³ and confined¹⁴ supercooled water can be accurately described as a two-step relaxation: a short-time Gaussian-type (in-cage vibrational) relaxation followed by a plateau and then a long-time (time > 1.0 ps) stretched exponential relaxation of the cage. The so-called relaxing-cage model (RCM),¹⁵ which we use for data analysis, models closely this two-step relaxation and has been tested extensively against bulk and confined supercooled water through MD and experimental data.^{13–15} By considering only the spectra with wave vector transfer $Q < 1.1 \text{ \AA}^{-1}$, we can safely neglect the contribution from the rotational motion of water molecule.¹⁵ The RCM describes the translational dynamics of water at supercooled temperature in terms of the product of two functions:

$$F_H(Q, t) \approx F_T(Q, t) = F^S(Q, t) \exp[-(t/\tau_T(Q))^\beta], \quad (1)$$

$$\tau_T(Q) = \tau_0(0.5Q)^{-\gamma}, \quad \langle\tau_T\rangle = \tau_0\Gamma(1/\beta)/\beta,$$

where the first factor, $F^S(Q, t)$, represents the short-time vibrational dynamics of the water molecule in the cage. This function is fairly insensitive to temperature variation and thus can be calculated from MD simulation. The second factor, the α -relaxation term, contains the stretch exponent β , and the Q -dependent translational relaxation time $\tau_T(Q)$, which strongly depends on temperature. The latter quantity is further specified by two phenomenological parameters τ_0 and γ , the exponent controlling the power-law Q -dependence of $\tau_T(Q)$. $\langle\tau_T\rangle$ is a Q -independent quantity where Γ is the gamma function. It essentially gives a measure of the structural relaxation time of the hydrogen-bond cage surrounding a typical water molecule. The temperature dependence of the translational relaxation time is then calculated from three fitted parameters, τ_0 , β , and γ , by analyzing a group of nine quasielastic peaks at different Q values simultaneously.

IV. RESULTS AND DISCUSSIONS

Figure 1 shows the mean-squared hydrogen atom displacements obtained by a method of elastic scan for hydrogen atoms in hydration water [$\langle x_{H_2O}^2 \rangle$], in DNA molecules ($\langle x_{DNA}^2 \rangle$), respectively. We obtained the latter using the D_2O hydrated sample, while the former using the difference spectra between the H_2O hydrated sample and the D_2O hydrated sample. Due to the presence of many hydrogen atoms in DNA, this subtraction is necessary. The fraction of the incoherent scattering signal from the hydrogen atoms in DNA is about 60% of the total incoherent scattering signal from H_2O hydrated DNA sample. One sees that at low temperatures up to their respective crossover temperatures, both curves have a gentle linear temperature dependence. But above the crossover temperatures, they both rise sharply with different slopes. We call the crossover temperature of the former T_L , and that of the latter T_C , both have values approximately 220 K. This shows that the dynamic crossover phenomenon of DNA and its hydration water is highly correlated, and occurs at the same temperature. As we shall see, this temperature can be defined much better for the hydration water in a dynamic measurement.

We show in Fig. 2, a temperature series of QENS area normalized spectra at $Q = 0.87 \text{ \AA}^{-1}$. Panel (B) shows the corresponding peak height as a function of temperature, whereas panel (C) is an enlargement of the wing area of the QENS spectra. Both these two panels give a visual information, before any data analysis, that there is an abrupt dynamical transition at $T_L = 220$ K.

Figure 3 shows the result of RCM analyses of the QENS difference spectra taken at $Q = 0.87 \text{ \AA}^{-1}$ for temperatures 230 K [panels (A1) and (A2)] 210 K [panels (B1) and (B2)], above and below the T_L , respectively. In this figure, we display the instrument resolution function purposely for comparison with the measured spectrum. RCM, as one can see, reproduces the experimental spectral line shapes of hydration

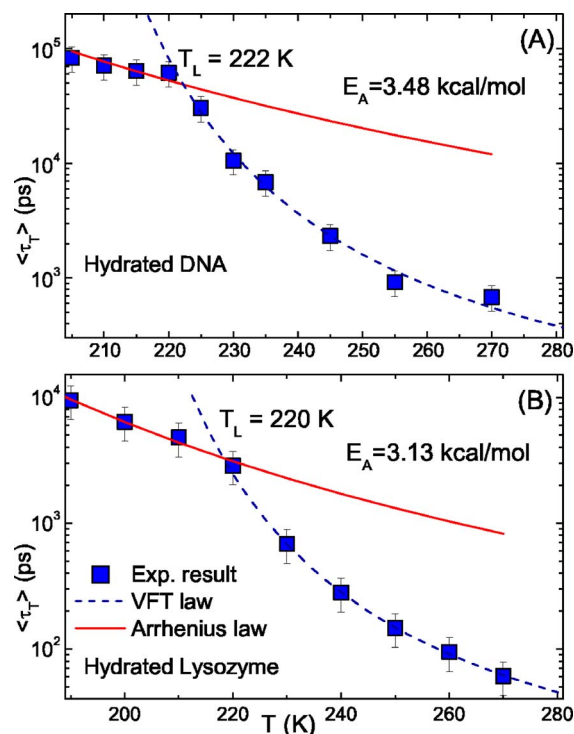


FIG. 4. The extracted Q -independent average translational relaxation time $\langle \tau_T \rangle$ from fitting of the quasielastic spectra plotted in log scale against temperature. Panel (A) is a result from hydrated DNA, whereas panel (B) is the same quantity measured in hydrated lysozyme shown for comparison (Ref. 9). There is a clear evidence in both cases a well-defined cusplike dynamic crossover behavior occurring at T_L indicated in the respective figures. The dashed lines represent fitted curves using VFT law, while the solid lines the fitting according to Arrhenius law. T_L in both cases occurs at 222 ± 2 K.

water quite well. The broadening of the experimental data over the resolution function leaves enough dynamic information to be extracted by RCM.

In Fig. 4, we present the temperature dependence of the average translational relaxation time, $\langle \tau_T \rangle$, for the hydrogen atom in a water molecule calculated by Eq. (1). It is seen that, in the temperature range from 270 to 230 K, $\langle \tau_T \rangle$ obeys Vogel-Fulcher-Tammann (VFT) law, a signature of fragile liquid, quite closely. But at $T=222$ K it suddenly switches to an Arrhenius law, a signature of a strong liquid. So we have a clear evidence of FSC in a cusp form. The T_0 for the fragile liquid turns out to be 180 K, and the activation energy for the strong liquid, $E_A=3.48$ kcal/mol. As a comparison, we plot the same quantity in panel B for hydration water in lysozyme protein.⁹ It is to be noted that the crossover temperature is sharply defined at $T_L=220$ K, slightly lower than in the DNA case.

In summary, we present unequivocal evidence that there is a fragile-to-strong dynamic crossover phenomenon observable in both DNA and protein hydration water. Above the crossover temperature, the hydration water is more fluid, implying having locally predominantly high-density water structure,^{16,17} with a not fully developed hydrogen-bond network; and below the crossover temperature, it evolves into locally predominantly low-density water structure, corresponding to an extensive hydrogen-bond network, which is

less-fluid. This mobility change across T_L can also be seen from the Q -dependence of ISF. We see from Eq. (1) that the product of exponent's β and γ gives the power-law Q -dependence of the ISF. Our result of the data analysis shows that at high temperatures, $\beta\gamma$ decreases steadily as the temperature decreases, reaching the lowest value of 0.2 at the crossover temperature of 220 K; then below this temperature, $\beta\gamma$ becomes essentially flat. It should be noted that for a freely diffusing water molecule, $\beta\gamma=2$, and if $\beta\gamma=0$, the ISF becomes Q -independent, meaning the water molecule is localized in space. Therefore the very low value of $\beta\gamma=0.2$ signifies a restricted mobility of the hydration water. There is a strong evidence from MD simulations of protein hydration water that this drastic change of mobility across the FSC triggers the so-called glass transition in protein molecules.^{18–20} This paper supplies an experimental evidence which reinforces this interpretation for the case of protein and DNA.²⁰

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